Supporting information

Biocompatible Polydopamine Fluoresecent Organic Nanoparticles: Facile preparation and cell imaging

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Experimental details

1. Materials and characterization

Dopamine hydrochloride was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). All other agents and solvents were purchased from commercial sources and used directly without further purification. Atomic force microscopy (AFM) images were taken out using a Nanoscope multiMode SPM (Digital Instruments) with a AS-12 scanner operated in tapping mode in conjunction with a V-shaped tapping tip (Applied Nanostructures SPM model: ACTA). Transmission electron microscopy (TEM) images were recorded on a TECNAIG2 20 microscope operated at 200 kV. The TEM specimens were made by placing a drop of the nanoparticle suspension on a carbon-coated copper grid. The images were recorded at a scan rate of 2 Hz. UV-Visible absorption spectra were recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz curettes of 1 cm path length. The fluorescence measurements were made on a PE LS-55 spectrometer equipped with quartz curettes of 1 cm path length.

2. Method

2.1 Preparation and characterization PDA-FONs

The preparation of PDA-FONs is shown in Scheme 1. First, dopamine (200 mg) was dissolved in about 40 mL of Tris buffered solution (10 mM, pH = 10.5) and stirred at room temperature (20 °C) for 15 min.[1] Then 10 mL of concentrated hydrogen peroxide H_2O_2 (30% w/w) was then added and reacted for another 5 h at room temperature. The obtained yellow solution was purified using dialysis tube (molecular weight cutoff ~ 1000 Da) and dialysis against deionized water and absolute ethanol, respectively. Finally, the purified solution was filtered with micropore filter (0.22 μm) and dried at under vacuum at 35 °C. The PDA-FONs were detailed characterized by a series of characterization techniques.

Quantum yield was measured according to established procedure the previous reports. The optical

densities were measured on UV-Vis spectra were obtained on a UV/Vis/NIR Perkin-Elmer lambda750 Spectrophotometer. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) was chose as a standard. Absolute values are calculated using the standard reference sample that has a fixed and known fluorescence quantum yield value, according to the following equation:

$$\varphi_\chi = \varphi_{std} \frac{I_x}{A_x} \frac{A_{std}}{I_{std}} \frac{\eta_x^2}{\eta_{std}^2}$$

Where φ is the quantum yield, I is the measured integrated emission intensity, and A is the optical density, and η is the refractive index. The subscript "std" refers to the reference fluorophore of known quantum yield. In order to minimize re-absorption effects absorbencies in the 10 mm fluorescence cuvette were kept under 0.1 at the excitation wavelength (360 nm).

The fluorescent stability of PDA-FONs was recorded on n a PE LS-55 spectrometer using time drive model. The excitation wavelength was set at 365 nm with a 10-nm slit width; the emission wavelength was set at 455 nm with a 10-nm slit width.

2.2 Observation of PDA-FONs using optical fluorescent microscopy

The tunable fluorescence of PDA-FONs was further evidenced by optical fluorescent microscopy. In brief, drops of PDA-FONs solution with the concentration of 200 μg mL^{-1} were dipped on a glass slide, and then a coverslip was put on the PDA-FONs drops. When observed the particles kept stationary under microscopy, both bright field and fluorescent images were taken by using a fluorescent microscopy (Leica, Germany). Three optical filters with different wavelength range were used to excite the samples. The filter names and wavelength ranges were listed below: filter A (λ = 340-380 nm), filter I3 (λ = 450-490 nm), filter N21 (λ = 515-560 nm).

2.3. Confocal microscopic imaging of cells using PDA-FONs

Mouse embryo fibroblast (NIH-3T3) cells were cultured in Dubecco's modified eagle medium

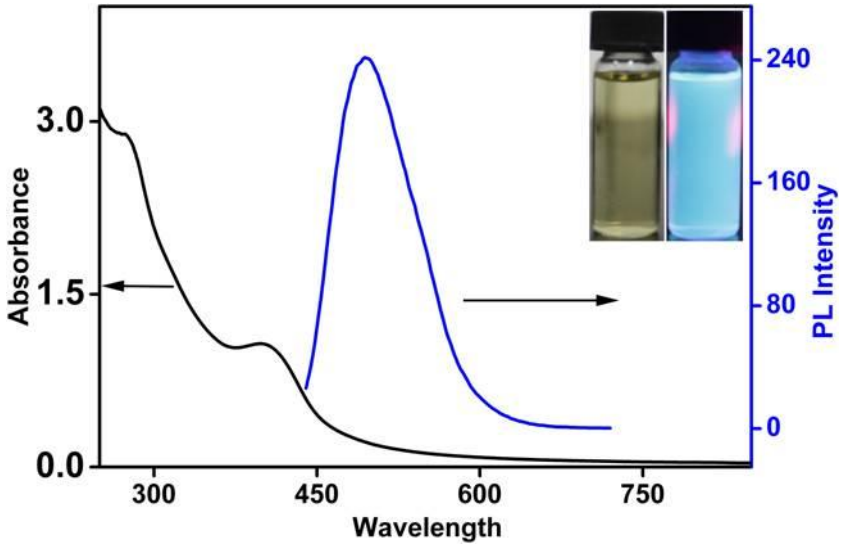
(DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every three days for maintaining the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with PDA-FONs at a final concentration of 150 μg mL^{-1} for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the PDA-FONs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a Laser Scanning Confocal Microscope (LCSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelengths of 405 nm and 458nm.

2.4. Cytotoxicity of PDA-FONs

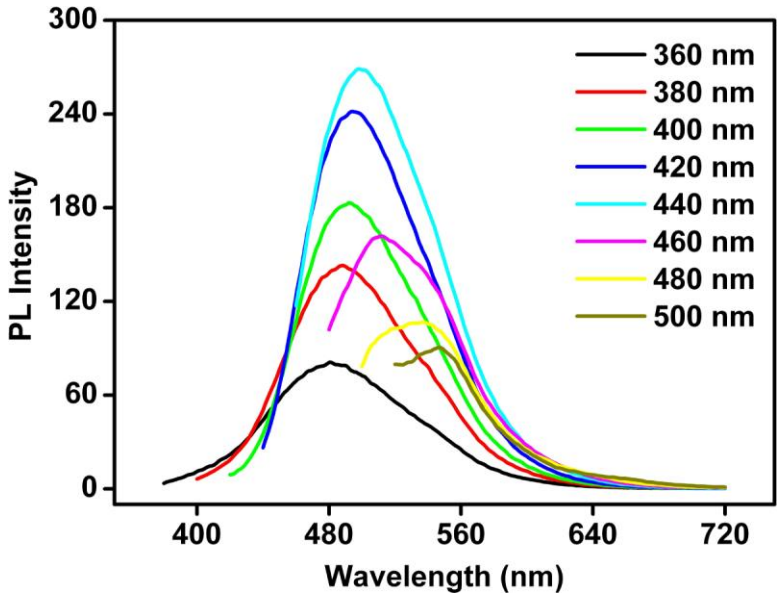
The cell viability of PDA-FONs on NIH-3T3 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports.[2] Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, 160 μg mL^{-1} PDA-FONs for 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μl of CCK-8 dye and 100 μl of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not exposure to PDA-FONs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance

relative to that of untreated controls. Results are presented as mean ± standard deviation (SD).

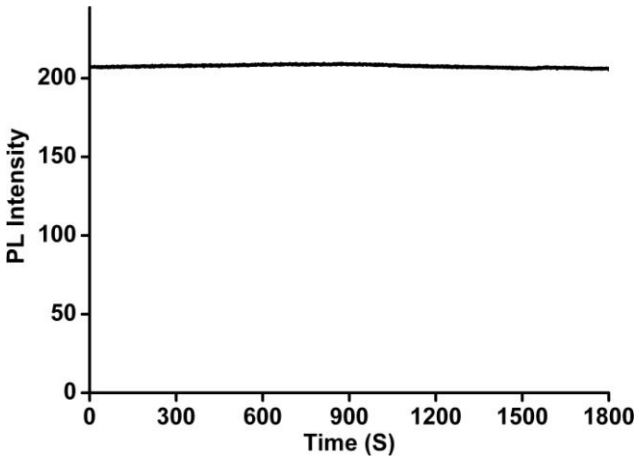
3. Results



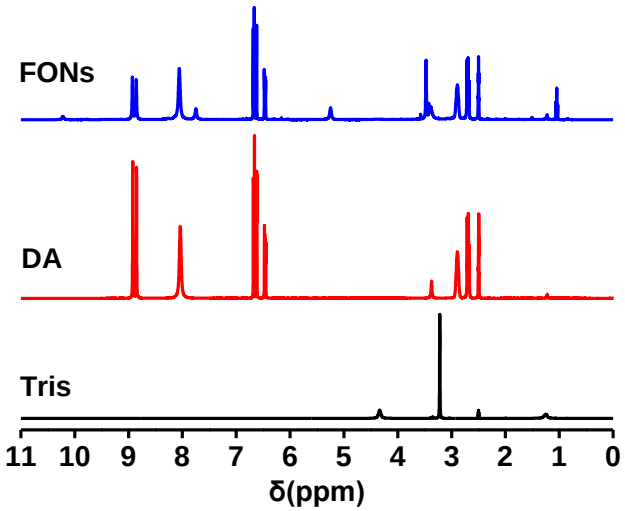
SFig. 1 UV-Vis absorption spectrum (black) and fluorescence spectrum (blue) of PDA-FONs dispersed in water; the exicited wavelength is 400 nm. Inset: photos of PDA-FONs dispersion (left) and under UV light at 365 nm (right).



SFig. 2 PL emission spectra (with radually increased excitation wavelengths from 360 nm to 500 nm of PDA-FONs dispersion in water.



SFig. 3 Emission intensity of PDA-FONs dispersion in water during continuous excitation at 365 nm.



SFig. 4 ^{1}HNM spectra of Tris, DA and FONs in DMSO.

References

1. S. Liu, J. Tian, L. Wang, Y. Luo, J. Zhai and X. Sun, *J. Mater. Chem.*, **2011**, *21*, 11726-11729.
2. X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Polymer Chem.*, Revision.